Arch. Biol. Sci., Belgrade, 64 (1), 159-174, 2012 DOI:10.2298/ABS1201159T

EVOLUTIONARY BIOLOGY STUDIES ON THE *IRIS PUMILA* CLONAL PLANT: ADVANTAGES OF A GOOD MODEL SYSTEM, MAIN FINDINGS AND DIRECTIONS FOR FURTHER RESEARCH

A. TARASJEV, S. AVRAMOV and DANIJELA MILJKOVIĆ

Institute for Biological Research "Siniša Stanković", University of Belgrade, 11000 Belgrade, Serbia

Abstract - Evolutionary studies on the dwarf bearded iris, *Iris pumila* L., a perennial clonal monocot with hermaphroditic enthomophylous flowers, have been conducted during the last three decades on plants and populations from the Deliblato Sands in Serbia. In this review we discuss the main advantages of this model system that have enabled various studies of several important genetic, ecological, and evolutionary issues at different levels of biological organization (molecular, physiological, anatomical, morphological and population). Based on published research and its resonance in international scientific literature, we present the main findings obtained from these studies, and discuss possible directions for further research.

Key words: Evolutionary biology, evolutionary ecology, ecological genetics, genetic variability, phenotypic plasticity, adaptations, model system, *Iris pumila,* natural populations, Deliblato Sands

INTRODUCTION

Good model systems for evolutionary studies are not easy to find. Evolutionary biology has developed through the mergers of a variety of disciplines, first by merging evolutionary theory with population genetics and then through the inclusion of other fields such as ecology, developmental biology, molecular biology and others; in the end all, biology converges in evolutionary biology (Futyuma, 1998). Specific features that are suitable for different experimental tasks in several different disciplines are usually not possessed by a single species. In addition, the subject matter of evolutionary biology is the remarkable diversity of organisms and their characteristics, so a comparative method is often used, which by definition involves more than one species. In evolutionary biology we are intrinsically interested in variations in the living world. Therefore, while in other areas of biology we usually have a large community of scientists all using small groups of organisms, in studies of evolution we have a relatively small group of scientists studying a very large number of organisms (Kellog and Schaffer, 1993). We cannot expect that any single species can assume the role of a model system for evolutionary biology comparable to the one that, for example, *Drosophila melanogaster* or *Arabidopsis thaliana* played and are still playing in the field of genetics. This is especially true for plants where no one plant can encompass the enormous diversity on the whole plant, physiologic, chemical, genetic, or molecular level (Mandoli and Olmstead, 2000). However, we can hope that by choosing appropriate model systems we should be able to answer more questions after painstakingly fine-tuning our experimental procedures for that particular species. In addition, we can hope that by choosing a good model system we can address additional questions that would involve different levels of biological or-

ganization and different evolutionary phenomena that might reasonably be interrelated.

This review presents three decades of research conducted in Serbia by various scientists on the dwarf bearded iris, *Iris pumila* L. which was utilized as a model system for evolutionary biology studies. In this work, we present its advantages and the main areas and different biological levels of organization covered in the performed research. We also review the main findings, relying on international publications that refer to work conducted on *I. pumila* and, finally, we have tried to suggest some promising directions for further research.

Iris pumila as a model system

The dwarf bearded iris *Iris pumila* L. is a small monocot that occurs in the lowlands of central and eastern Europe (Mathew, 1981). It was chosen for ecological genetics and evolutionary ecology studies at the beginning of 1980s by Dr Branka Tucić, a researcher from the Institute for Biological Research of the University of Belgrade, and Prof. Dr Vladimir Stevanović from the Faculty of Biology of the University of Belgrade. Over the decades that followed, it proved to be an excellent choice for this type of research for a number of reasons.

Firstly, *I. pumila* possesses large, hermaphroditic, enthomophylous flowers (Fig. 1), that can be hand-pollinated easily. Many genetic experimental designs can therefore be applied through controlled pollinations and they may include reciprocal crossings (the same plant serving both as male and female) that can be used for the study of several genetic and non-genetic effects (maternal, cytoplasmic, epigenetic etc.). Controlled pollination of *I. pumila* can be ensured if the right procedure is followed. Perianth parts that serve as landing sites for bumblebees (namely the falls) can be removed in order to prevent any outcrossing other than those desired and personally performed. *I pumila* is a strictly outcrossing species (Tarasjev, 2005), so the seed set from controlled pollinations will not be contaminated by selfing. The only disadvantage is that the flowers last for only a short period of time and flowering that occurs in early spring (April) ends in a matter of weeks.

Fruits (capsules) of *I. pumila* mature in mid-June and the seeds are large and easy to manipulate, although seed dormancy must be broken by drastic methods in order to ensure simultaneous germination. On the other hand, dormancy presents a research opportunity since it can be studied as a phenomenon on its own, and it leads to "seed banks" in the soil that can also be analyzed.

I. pumila is a perennial clonal plant that spreads vegetatively through underground rhizomes. Therefore, the same genotypes can be studied over different years and a sufficient number of replicas of the same genotypes that are established in natural populations can be obtained for experimentation. This is particularly important from the conservation standpoint since sample collection procedures will not involve removing whole genotypes from natural populations so that the population structure and diversity can be protected. The same naturally occurring genotypes can therefore be studied over different environments, multifactorial natural or single factor experimental settings. While single factor experiments have the benefit of controlling variation to a very high degree and have a high analytical power, experiments in natural environments have an advantage in the utilization of naturally occurring variations and are therefore far more realistic (Sultan, 2001).

The importance of analyses of genetic variability in evolutionary biology studies cannot be overemphasized, and *Iris pumila* exhibits huge flower color genetic polymorphism whose maintenance is a very interesting evolutionary problem on its own. This polymorphism also enables easy identification of genetically distinct clones in the wild (Fig. 2). Moreover, first pilot studies revealed significant genetic variability compared to other *Iris* species (Tucić et al., 1984a, 1984b).

I. pumila inhabits the protected natural reserve of the Deliblato Sands (44°48'N, 20^0 58' E) which are

Fig. 1. Some of the flower color variants in *Iris pumila* L.

located approximately 50 km northeast of Belgrade, Serbia. The Reserve is a good source of undisturbed natural populations that can be monitored and surveyed for the consequences of so-called "natural experiments" and from which representative samples can be collected. It is also a good place for the setting of small-scale experiments that won't be disturbed. In the Deliblato Sands, *I. pumila* can be found in quite different habitats. The most common are open habitats with steppe-type vegetation (Stjepanović-Veselčić, 1953), but it can also be found on forest edges which have a completely different set of ecological indices compared to the open ones, the most profound of which is the light regime that differs not only in the quantity but also in the quality of light

Fig. 2. *Iris pumila* clones in their natural environment during the flowering peak

Table 1. Analyzed phenomena in evolutionary biology studies on *Iris pumila* L.

Phenomenon	Studied in
Genetic variability	Tucić et al. 1984a, 1984b, 1988, 1989, 1990, 1998, 2003 Tarasjev 1989, 1995a, 1997, 2002, 2003, 2005a, 2005b, Pemac and Tucić 1998, Tarasjev et al. 2006, 2009, Avramov and Tucić 2006, Avramov et al. 2007, Manitašević 2010, Živković 2011, Manitašević et al. 2011
Phenotypic plasticity.	Tarasjev 1989, 1994, 1995a, 1995b, 1997, 2002, 2003, 2005a, 2005b, Tucić et al. 1990, 1998, 2003, 2009, Pemac and Tucić 1998, Tarasjev et al. 2006, 2009, Avramov and Tucić 2006, Avramov et al. 2007, Manitašević et al. 2007, 2011, Miljković 2008, , Vuleta and Tucić 2009, Manitašević 2010, Vuleta et al. 2010,
Developmental instability	Tarasjev 1995a, 1995b, Tucić et al. 2008, Miljković 2009, 2011, Manitašević 2010, Tucić and Miljković 2010,
Between population differentiation	Tarasjev 1989, 1994, 1995a, 1995b, 1997, 2002, 2003, 2005a, 2005b, Pemac and Tucić 1998, Tucić et al. 1998, 2003, 2005, Tarasjev et al. 2006, 2009, Avramov and Tucić 2006, Avramov et al. 2007, Manitašević 2010, Tucić and Miljković 2010, Manitašević et al. 2011[4],
Maternal effects	Tucić and Avramov 1996a
Environmental stress	Manitašević et al. 2007, Tucić et al. 2008, 2009, Miljković 2009, 2011 Vuleta and Tucić 2009, Vuleta et al. 2010, Manitašević 2010, Tucić and Miljković 2010, Živković 2011,
Phenotypic selection	Tucić et al. 1998, 2005, Avramov et al. 2007
Reproductive success (vegetative and sexual), and survival	Tucić 1993, Tarasjev 1995a, 1997, 2002, 2005a, 2005b, Tarasjev et al. 2009

(vegetation shade with an altered red/far red ratio). This enables various studies involving populations from different habitats and the utilization of such diverse natural environments.

Analyzed topics and traits

The evolutionary research on *Iris pumila* has been published in more than 50 bibliographic units (for references until 2004 see Tarasjev, 2005c), including three M. Sci theses (Tarasjev, 1989; Avramov, 1995; Pemac, 1997) and four PhD theses (Tarasjev, 1995a; Avramov, 2007; Miljković, 2009; Manitašević, 2010). Research has concentrated mostly on evolutionary ecology (studies on how the ecological characteristics of the species evolve) and on ecological genetics (how the genetic constitution of populations is affected by environmental factors). The most analyzed single topic in these studies was phenotypic plasticity (Table 1), i.e. the ability of a genotype to phenotypically respond to changes in environmental conditions that can, under certain conditions, evolve as an adaptation to heterogeneous environments (Schlichting and Pigliucci, 1998; Pigliucci, 2001). Also, genetic variability and between-population differentiation were addressed in a great number of studies of *I. pumila*. They are of great interest to evolutionary studies *per se*, as they represent the basis for evolution by natural selection and are the first steps in evolutionary divergence, but their relationship with phenotypic plasticity is of great importance and interest as well. It was hypothesized that the relationship between plasticity and genetic variability could be negative since they represent "alternative ways of dealing with environmental heterogeneity" and that phenotypic plasticity could act as a "buffer against selection" and therefore inhibit evolutionary change. Others have argued that phenotypic plasticity could facilitate evolutionary change in various ways (Schlichting and Pigliucci, 1998; Pigliucci, 2001). Analyses of phenotypic selection (Tucić et al., 1998; Avramov et al., 2007) were directly related to selection regimes under different environmental setups and to evolutionary potential, as well as studies of reproductive success un-

Table 2. Analyzed traits in evolutionary biology studies on *Iris pumila* L.

Trait group	Traits	Studied in
Molecular and physiological	1.Isoenzyme polymorphism, 2.Chlorophyll and its derivatives, 3.Heat shock proteins 4.Anthocyanins 5. Heavy metal content	Tucić et al 1984a[1], Tucić et al 1984b[1], Avramov and Tucić 2006[2], Avramov et al 2007[2], Manitašević et al 2007[3], Tucić et al 2008[3], Miljković 2009[1], Tucić et al 2009[2,4], Vuleta and Tucić 2009[1], Vuleta et al 2010[1], Manitašević 2010[3],Manitašević et al 2011[3], Živković 2011[5]
Anatomical	1.Stomatal density 2.Leaf thickness 3.Vascular bundle number 4. Schlerenchima width 5.Cuticle width	Avramov and Tucić 2006[2,3,4,5], Avramov et al 2007[1,2,3,4,5]
Morphological	1.Flower parts size 2. Leaf size 3. Specific Leaf Area 4.Rhizome size	Tarasjev 1989 [1,2,4], Tucić et al 1990 [1,2], Tarasjev 1994[1,2], Tarasjev 1995a[1,2], Tarasjev 1995b[1,2] Tucić et al 1998[2], Tarasjev et al 2006[1,2] Manitašević et al 2007[3], Avramov et al 2007[3], ,Tucić et al 2008[1], Tarasjev et al 2009[1,2] Tucić and Miljković 2010[1] , Manitašević 2010[32,3], Miljković 2011[1]
Life history	1.Germination time, 2.Leaf dynamics, 3.Size during development 4.Flowering time 5.Mortality and survival	Tarasjev 1995a [1,2,4], Tarasjev 1997[4], Pemac and Tucić 1998[1,2,3], Tucić et al 2003 [2], Tucić et al 2005[2,3] Tarasjev 2005b[5]
Reproductive	1.Number of ramets, 2.Number of flowers 3.Fruit number 4.Fruit to flower ratio	Tarasjev 1995a [2,3,4] Tarasjev 2002[1] Tucić et al 2003[1], Tucić et al 2005[1], Tarasjev et al 2009[2,3,4]

der natural conditions (Tarasjev, 2005a). Analyses of developmental instability (Tarasjev, 1995b) were also conducted to shed light on its relationship with phenotypic plasticity since it was hypothesized that both plasticity and instability should increase with decreased levels of heterozygosity, but this relationship was questioned (see Schlichting and Pigliucci, 1998 and references therein). Maternal effects were also investigated as they can also be evolutionarily altered, but they can also influence the potential for evolutionary response to the selection of early seedling traits (Tucić and Avramov, 1996). Finally, while a plethora of studies necessarily included elements of stress resulting from extreme treatments of the utilized spectrum or even just in an alternative natural environment, specific research on environmental stress were also carried out (Table 2) and responses to it were studied at different levels of biological organization (Manitašević et al., 2007).

Other evolutionary biology studies of *I. pumila* also utilized traits from various levels of organization: from molecular and physiological, anatomical and morphological, to life history and reproductive traits (Table 2). While molecular and physiological levels were present from the very start of work on *I. pumila* through electrophoretic analyses of isoenzyme polymorphism (Tucić et al., 1984a, 1984b), in recent times it was expanded to important biological molecules such as chlorophyll (Avramov and Tucić, 2006), heat shock proteins (Manitašević et al., 2007) and anthocyanins (Tucić et al., 2009).

Main findings

The main findings in research performed on *I. pumila* in the last three decades have had an impact on the international scientific community and have been referred to in several papers as well as in influential monographs or reviews of these topics (Møller and Swaddle, 1997; Schlichting and Pigliucci, 1998; Pigliucci, 2001; Palmer and Strobeck, 2003; Roff and Fairbairn, 2007; Schlichting, 2008; Vallejo-Mar et al., 2010; Donovan et al., 2011).

Table 3. Main findings in evolutionary biology studies on *Iris pumila* L. according to sources in international publications

Paper	Topics	Main findings	According to
Tucić *et al* 1990. *Plant. Sys. Evol*. 170, 1-9.	Intraclonal and interclonal morphological variability in three natural microhabitats	Environmental heteroscedascity detected. Indication of no inverse relationship between plasticity and genetic variability	Obeso and Herrera 1994. Andersson 1995. Dutilleul and Potvin 1995. Back et al 1996. Schlichting and Pigliucci 1998. Sapir et al 2002
Tarasjev 1995. *Russ.J. Genet.* 31:1655-63	Comparison of five levels of morphological variability	Developmental instability and phenotypic plasticity are not correlated on the same organisms	Møller and Swaddle 1997. Schlichting and Pigliucci, M. 1998. Cuervo and Moller 1999. Roy and Stanton 1999. Pigliucci, 2001, Esser et al 2002. Palmer and Strobeck, 2003. Kristensen et al 2003 Van Kleunen and Fisher 2005
Tarasjev 1997. *Ecography* 20:48-54	Phenotipic plasticity and between population differentiation for flowering phenology	Phenological responses to temporal and spatial variation in climatic conditions are maintained by natural selection. Plasticity and population differentiation had the same direction	Price, and Wasser 1998. Sultan 2001 Pico and Retana 2001. Torres et al. 2002. Buide et al. 2002. Martinkova et al. 2002. Roncal, 2007. Tagane, 2007. Andrew and Ustin 2009. Atlan et al. 2010.
Tucić et al 1998. *Acta Oecol.* 19:473-481	Phenotypic selection	Cost of the plasticity has been detected	Schlichting and Smith. 2002, Navas and Gsarnier 2002, Steinger et al 2003. Ernande and Dieckmann. 2004, Lepik et al. 2004. Franiel and Więski 2005, Tamás, J. & Hably, L. 2005, 2009, Bell and Galloway 2008. Van Buskirk and Steiner 2009. Ramírez-Valiente et al 2010. Donovan et al 2011
Tarasjev 2005. *Acta Oecol* 27: 93-98.	Clone size and female reproductive success	Large clones had smaller fruit to flower ratio	Kostrakiewicz 2007, Monty et al. 2006. Cottrell et al 2009. Liao et al. 2009, Vallejo-Mar et al. 2010
Manitasević et al 2007 *Plant Cell Environm*, 30: 1-11	Seasonal and habitat variation in heat shock proteins Hsp70 and Hsp90 expression	Role of heat shock proteins in buffering phenotypic variation in the wild.	Tonsor et al 2008, Schlichting 2008, Henkel and Hofmann 2008 Henkel et al 2009, Fu 2009
Avramov et al 2007 *Plant Ecol.* 190: 275-90	Adaptive plasticity in irradiance gradient of morphological, anatomical and physiological traits	Adaptive plasticity to irradiance level demonstrated	Van Kleunen and Fischer 2007, Van Buskirk and Steiner 2009, Lind and Johansson 2009, Zhao et al. 2010, Loranger and Shipley 2010, Ramirez-Valiente et al. 2010, Baythavong and Stanton 2010, Donovan et al. 2011, Pelabon et al 2011

In the evaluation of the main findings of evolutionary biology studies on *Iris pumila* L, in addition to published research papers and papers with a theoretical background of performed research, we can also utilize the interpretations and suggested implications of those results that can be found in scientific literature. The studies of *I. pumila* that had the most impact on the work of other researchers are presented in Table 3.

The first studies of evolutionary biology issues on *Iris pumila* focused mostly on estimations of its genetic variability in populations. They included surveys of isoenzyme polymorphisms and its comparison with selected congeneric species (Tucić et al., 1984a, 1984b), as well as estimations of clonal diversity on spatial and temporal scales (Tucić et al., 1988, 1989). These studies served as an extremely important starting point, and the obtained electrophoretic data served as a baseline for isoenzyme variability evaluation for other species of Iris sect. Iris (Arafeh et al., 2002).

Another important finding arising from early studies on *Iris pumila* is the documentation of among-environment heteroscedasticity (Dutilleul and Potvin, 1995) – differences in within-environmental variances between environments that were obtained from the study of the within- and between-clone variability of flower morphometric traits in three microhabitats – top, slope and depression between dunes (Tucić et al., 1990) (Table 3). Of significant importance was the fact that this study found no negative relationship between within- and among-clone variability. This indicated the possibility of no negative relationship between genetic variability and phenotypic plasticity (Schlichting and Pigliucci, 1998) – the negative relationship that should be expected if genetic variability and phenotypic plasticity are alternative ways of dealing with environmental heterogeneity. This indication was later confirmed for the same traits in the analysis of transplant/replant experiments where genetic and environmental components of variability were strictly separated (Tarasjev, 2003).

One of the very important issues regarding the evolutionary role of phenotypic plasticity is its relationship with developmental instability. It was hypothesized that there is a positive relationship if they share the same genetic basis and both are the result of developmental noise, and also that there should be no relationship if plasticity is a trait on its own and in some cases adaptive (Pigliucci, 2001). A significant contribution to addressing this problem came from the analysis of different levels of morphometric variability of *Iris pumila* flower parts (Tarasjev, 1995b) (Table 3). The analysis included two types of phenotypic plasticity (spatial and temporal), two types of variability that are the result of both microenvironmental variability and developmental noise (between flowers on the same transplant/replant and between replicas of the same genotype in the same environment), and flower asymmetry that is solely the outcome of developmental instability. This original experimental setup in the *I. pumila* study was considered as an appropriate design for addressing these issues (Pigliucci, 2001). Analysis of the correlation of those measures on the same individual genotype revealed that phenotypic plasticity and developmental instability were not correlated and this was reflected in several important publications on the issue (Møller and Swaddle, 1997; Schlichting and Pigliucci, M. 1998; Cuervo and Moller, 1999; Roy and Stanton, 1999; Pigliucci, 2001; Palmer and Strobeck, 2003). In a couple of other studies of *I. pumila*, developmental instability was also investigated as a trait on its own, and its genetic variability, habitat specificity and relationship with heat shock proteins (HSP) were analyzed (Tucić et al., 2008, Miljković, 2009, 2011, Manitašević, 2010, Tucić and Miljković, 2010). The statistical association between HSPs as molecules with a known buffering capacity, and developmental instability, was not found in these studies (Tucić et al., 2008), while between-trait variation in developmental instability (measured by asymmetry) was detected (Tucić and Miljković, 2010, Miljković 2011)

While phenotypic plasticity has received the greatest attention in evolutionary ecology research on *I. pumila*, between-population differentiation

was also a topic in several studies (Table 2). Tarasjev (1997) utilized a transplant/replant experiment in *Iris pumila* natural environments (open dune and shaded forest habitats) to simultaneously approach both phenotypic plasticity and between-population differentiation in flowering phenology (Table 3). Since ecological differences between habitats are complex rather than single-factor in nature, such data for the natural multifactorial environments of *I. pumila* offer greater insight (Sultan, 2001). This study detected differences in the flowering time between open and shaded habitats (with shaded habitats flowering later). Later flowering in a more shaded habitat was detected in some subsequent studies on other species, but instances where flowering in a shaded habitat started earlier were observed as well (Andrew and Ustin, 2009). The observed difference in flowering time was, to a lesser degree, maintained in experimental plots and all observed patterns were stable over years. The environmental aspect of phenology was therefore evident in responses to temporal and spatial variation in *Iris pumila* phenology (Price and Wasser, 1998). These observations also suggest that a species phenological position in the growing season, and its phenological response to environmental cues, are maintained by ongoing natural selection (Price and Wasser, 1998). The fact that the phenotypic variation observed in natural populations was maintained in experimental plots, demonstrates genetic differentiation in *Iris pumila* (Atlan et al., 2010). A similar pattern of relationship between phenotypic plasticity and between-population differentiation was also detected in the same transplant/replant setting for flower and leaf morphological characters (Tarasjev et al., 2009).

In other studies that addressed phenotypic plasticity, Pemac and Tucić (1998) investigated reaction norms of *I. pumila* juvenile traits to light intensity using slope of reaction norm vs. environment as a measure of plasticity and plant biomass as a covariate (Valladares et al., 2006), while Tucić et al. (2003) investigated plastic responses to the photoperiod of *I. pumila* multiple leaf phenological traits (Wolfe and Mazer, 2005). It is important for evaluating the evolutionary possibilities for *I. pumila* coping with environmental change through phenotypic plasticity that Pemac and Tucić (1998) found little evidence for heritable variation in environmental sensitivity (Wolfe and Mazer, 2005)

Several studies of *I. pumila* estimated selection differentials and gradients and evaluated the costs of plasticity and/or its tradeoffs (Tucić et al., 1998, Tucić et al., 2005, Avramov et al., 2007). Although the costs of phenotypic plasticity as potential constraints for the evolution of phenotypic plasticity were frequently mentioned for a long time, Tucić et al. study (1998) (Table 3) was among the first experimental attempts to evaluate these costs in plants (Steinger et al., 2003, Ernande and Dieckmann, 2004; Bell and Galloway, 2008), and a limited cost of plasticity was found. This study also provided evidence of a larger variability in leaf number and distribution (i.e. whole plant traits) than in leaf traits (i.e. metamer traits) in response to environmental changes (Navas and Gsarnier, 2002). It also provided evidence that light availability strongly affects the plant above-ground biomass distribution pattern and shoot height (Lepik et al., 2004) and that at least some of the morphological leaf characteristics can be subject to considerable modification in response to varying environmental conditions (Franiel and Więski, 2005). According to Tamás and Hably (2009), this study also revealed that higher light intensity results mainly in the formation of smaller and thicker leaves. According to Zhao et al. (2010), the study of Avramov et al. (2007) (Table 3) demonstrates that remarkable phenotypic variation could be observed among individuals of the same genotype developing in different habitats. It also shows adaptive plasticity in response to discrete variation in irradiance level (Baythavong and Stanton, 2010) that was most similar to the differences observed between "sun" and "shade" leaves (Pelabon et al., 2011), as well as the costs of plasticity (van Kleunen and Fischer, 2007). This study also showed that for the same factor specific leaf area (SLA) and stomatal density respond in different directions (Loranger and Shipley, 2010). The study of Avramov et al. (2007) was included in a meta-analysis of selection differentials and gradients done by Van Buskirk and Steiner (2009). Similar to the situation in many other

"hot topic" areas in evolutionary biology, some of the interpretations of findings on *Iris pumila* prompted controversy, as in the case of the Tucić et al., (2005) study that tested the predictions of an evolutionary trade-off model. This study was discussed and criticized by Roff and Fairbairn (2007), the main issues being the possibility of previous environmental conditions affecting allocation patterns and the need to differentiate between expectations from comparison between regressions observed under different environmental conditions and regressions observed for different populations grown under the same environment.

Exploiting the clonality of *Iris pumila* and the huge flower color polymorphism that enables the detection of distinct clones in the wild, Tarasjev (2005) (Table 3) studied the dependence of female reproductive success on clone size and within-clone flowering time. According to Liao et al. (2009), this study is of only a handful of studies to date that have addressed the influence of clone size on maternal success. According to the results, *I. pumila* genotypes are self-incompatible and female reproductive success was reduced by a number of simultaneously opened flowers of the same genotype. In large clones with many opened flowers there is an increased probability of pollen transfer between flowers of the same genetic individual (i.e. geitonogamy), which in self-incompatible species such as *I. pumila* can lead to a reduction in the proportion of flowers which set fruit due to stigma saturation and compatible pollen limitation (Cottrell et al., 2009). This finding is of great importance for studies of polymorphism maintenance in *I. pumila* populations.

Utilizing one of the advantages of *Iris pumila* as a model system (hermaphroditic flowers), Tucić and Avramov (1996a) studied maternal effects on early juvenile traits by carrying-out reciprocal crosses. As was pointed out by Imbert (1999), these results showed that non-genetic maternal factors affected plant performances, including early seedling traits that are not correlated to seed mass. In another study, Tucić and Avramov (1996b) further expanded the research area and analyzed morphological integration in *Iris pumila*. Their results supported the conjecture that phenotypic (Pearson product-moment) correlations can be a good substitute for genetic correlations (Reusch and Blanckenhorn, 1998), and also, according to Badyaev and Foresman (2000), suggested a long historical persistence of integrated functional complexes that can be expected in cases of environmental and genetic canalization due to persistent stabilizing selection.

A very important field of evolutionary studies of *Iris pumila* that is increasing in recent years, are studies at the molecular and biochemical levels. Manitašević et al. (2007) (Table 3) quantified Hsp70 and Hsp90 expression variation in response to season and habitat in *Iris pumila*. Differences were seen among samples collected from different habitats or seasons, with plants growing in open habitats exhibiting more Hsp70s than samples collected from shaded areas. These findings are extremely important since Hsp70s are a kind of potential biomarker of environmental stresses and can be applied to monitor environmental conditions (Fu, 2009). This is especially true in plants, since non-mobile organisms are especially interesting because they are unable to escape from unfavorable conditions (Henkel and Hofmann 2008). We still know little about naturally occurring variations in HSP or any resulting phenotypic or fitness effects in any species (Tonsor et al., 2008), but in buffering phenotypes against environmental variation, HSPs can also hide genetic variation (Schlichting, 2008). Therefore, dysfunction in HSP expression can uncover hidden genetic variations on which selection might act in periods of high stress (Tonsor et al., 2008). Analyses of HSP were also carried out in studies by Tucić et al. (2008) and Manitašević et al. (2011). Other analyses of *Iris pumila* in the evolutionary context on molecular and physiological levels included chlorophyll and its derivates (Avramov and Tucić, 2006, Avramov et al., 2007, Tucić et al., 2009), and recently, anthocyanins (Tucić et al., 2009) and several important enzymes (Vuleta and Tucić, 2009, Vuleta et al., 2010).

The most important findings obtained in almost thirty years of studying the dwarf bearded iris *Iris*

pumila L from an evolutionary perspective were primarily related to phenotypic plasticity that was studied on molecular, biochemical, anatomical, morphological and population levels. It has been shown that phenotypic plasticity can be adaptive and selected, that its genetic basis is different from the genetic basis of developmental instability, that it has costs, and that plasticity is not necessarily antagonistic either to genetic variability within or to genetic differentiation between populations. The performed research also showed that maternal effects can be a frequent phenomenon, that the concept of "generalized asymmetry" may not be correct, and that the correlation structure can change in different environments and different stages of development. The main advantage of these studies is that they were performed on natural populations in natural conditions or on samples from natural populations under experimental settings that utilized ecologically relevant environmental variations. It enabled insights into naturally occurring variability, from the assessment of the variability of biologically important molecules such as heat shock proteins and chlorophyll, to the estimation of phenological patterns and the spatial structure of variability in the wild. This multi-year research on *Iris pumila* has also provided valuable information and methodology for other researchers working on similar problems or species. In addition, information about *I. pumila* diversity in the Deliblato Sands, and about plastic responses and adaptive values under different naturally occurring environmental conditions, can be valuable in programs of dwarf bearded iris conservation as well as for biomonitoring. Finally, the performed studies paved the way for determining the most promising directions for further evolutionary research on *Iris pumila*.

Directions for further research

Research performed over three decades has resulted in many important findings that can be built upon. In our opinion, the main and most promising directions for further research include integrative studies, studies of the maintenance of genetic variability in populations, long-term population studies of reproduction and survival, and studies of environmental stress, especially those that have clear relevance for biomonitoring and the conservation of diversity.

Integrative research

Lines of research that can be followed under this direction include the integration of different biological levels of organization, different developmental stages and different sets of traits in the same analyses. These lines were already present in previous work. Detailed, specific data accumulated in previous studies enables researchers to approach those issues more efficiently. Different levels were previously utilized as in the study of the relationship between heat shock proteins and morphological instability measured by asymmetry (Tucić et al., 2008). The approach that involves different groups of traits was already used in studies that included both anatomical and physiological traits (Avramov, 2007). New features that have been used in the most recent studies, such as anthocyanins (Tucić et al., 2009) or additional enzymes (Vuleta and Tucić, 2009, Vuleta et al., 2010) expand these possibilities even further. The integrative approach is simplified by the information we have gained in many previous studies regarding traits that are the best representatives of whole sets of traits. For example, previous research has clearly shown that twenty analyzed morphometric flower traits can be substituted by fewer ones, with stem length as the most suitable candidate to represent morphological response to vegetation shade (Barišić Klisarić et al., 2011). Integration of the different stages in the *I. pumila* life history is already shedding more light on the different constraints for anatomical and physiological trait evolution in seedlings and mature plants (Miljković et al., 2011)

Maintenance of genetic variability

One of the most astonishing features of *Iris pumila* is its huge flower color polymorphism that can exist in a single population and/or locality, e.g. Tucić et al. differentiated nine distinct color morphs in a single population in their 1988 study. While this polymor-

phism was one of the main advantages of this model system that enabled the identification of genotypes in the wild, it will be a highly rewarding task to utilize it for answering the important evolutionary question about the mechanisms of genetic variability maintenance in natural populations. Natural selection is expected to deplete genetic variability and rare morphs can also be eliminated from populations by genetic drift, so some mechanism is needed to account for the maintenance of polymorphism, with some form of balancing selection as the most obvious candidate (Futyuma, 1998). In the case of *Iris pumila* that possesses both sexual and vegetative modes of reproduction with overlapping generations, research must include simultaneous and long-term studies of the evolutionary factors acting in populations. This work is at the very beginning, with some attempts to relate cohort flower diversity to climatic factors (Tucić et al., 1988) or to relate female reproductive success to vegetative propagation success measured by clone size (Tarasjev, 2005). In the case of flower color polymorphism, it must also involve detailed studies of the exact genetic basis of this diversity.

Long-term population studies

Another important direction of research is long-time population studies. This research could reveal the evolutionary mechanisms acting in *I. pumila* populations that enable not only the maintenance of genetic variability but also the very persistence of populations in their natural habitat in the face of changing environments and vegetative succession. It must involve studies of sexual and vegetative reproduction, clonal survival, and diversity preserved in soil seed banks. Previous results that involve the possible effects of climatic factors on diversity (Tucić et al., 1988) and possible interplay between sexual and vegetative reproduction (Tarasjev, 2005a), must be followed by studies that will involve male reproductive success, pollinator choice studies and ramet dynamics in the wild (this was studied in experimental conditions by Tarasjev, 2002). Since *I. pumila* is classified as an endangered species, these studies will also have a significant impact on conservation strategies.

Environmental stress, biomonitoring and conservation

While most of the studies conducted on *Iris pumila* have involved stressful conditions in the more extreme parts of utilized environmental gradients, studies that specifically focus on environmental stress should be the subject of future research. Good examples of this direction are studies that involve heat shock proteins (Manitašević et al., 2007), which in some instances simultaneously involved different levels (biochemical and morphological ones in Tucić et al., 2008).

Destructive human activities, such as the contamination of environments or destruction of habitats, calls for an all-out effort to conserve biodiversity, and the rapidly developing field of conservation biology makes extensive use of evolutionary principles (Futyuma, 1998). Future research performed on *I. pumila* should also focus on this applied component. Conservation strategies depend mostly on long-term population studies yet to be conducted, and there have only been first steps in this direction involving studies of reproductive success, ramet dynamics and mortality (Tarasjev, 2002, 2005b). On the other hand, regarding the use of *I. pumila* in the detection of air pollution both through the estimation of heavy metal accumulation and assessment of the levels of developmental instability, as well as by estimation of its genetic variability and susceptibility to other environmental factors, some work has already been done and some is in progress (Živković et al., 2011). This research can lead to the utilization of *I. pumila* as a bioindicator of the early phases of possible pollution in their natural protected habitats.

CONCLUSIONS

Evolutionary biology studies of the dwarf bearded iris, *Iris pumila* L. (Iridaceae), were conducted in the last three decades on plants and populations of this perennial clonal monocot with hermaphroditic flowers from the Natural Protected Reserve of the Deliblato Sands, with special emphasis on evolutionary ecology and ecological genetics. The main

advantages of this model system are the existence of both clonal and sexual reproduction that enables the implementation of various experimental designs, including various crossings (full-sib, half-sib and reciprocal, among others), as well as transplantation/replantation ones, huge flower color polymorphism that enables the identification of genetically distinct clones even on small distances, and the significant amount of genetic variability both within and among populations. These characteristics enabled various studies of *I. pumila* adaptations to environmental heterogeneity, its phenotypic plasticity and developmental instability, as well genetic variability and between-population differentiation. Research was conducted on a great variety of *I. pumila* traits, including phenological, morphological, life history and physiological ones at various stages of the life cycle of *I. pumila* development ranging from seeds and seedlings up to the adult flowering phases. Results obtained in these studies addressed several important genetic, ecological, environmental and evolutionary issues that were reflected in influential publications of other authors. These studies also provided insights and directions and can serve as an important basis for future research that could focus on integrative research, studies of genetic variability maintenance and long-term population studies, as well as on analyses of environmental stress and studies that can lead to practical applications of evolutionary findings for biomonitoring and conservation purposes.

Acknowledgement - This work was funded by the Ministry of Education and Science of Republic of Serbia (project OI 173025 "Evolution in heterogeneous environments: mechanisms of adaptation, biomonitoring and conservation of biodiversity"). English language reader: dr Krinka Vidaković Petrov.

REFERENCES

Andersson, E. (1995). Age-related morphological differentiation among populations of *Dactylorhiza traunsteineri* (Orchidaceae) in eastern Sweden. *Nord. J. Bot*. **15**(2): 127-37.

Andrew , M. E., and *S. L. Ustin* (2009). Effects of microtopography and hydrology on phenology of an invasive herb. *Ecography* **32**, 860-870

Arafeh, R. M., Sapir,Y., Shmida, A., Iraki, N., Fragman , O., and *H. P. Comes* (2002). Patterns of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (Iris sect. Oncocyclus = the royal irises) along an ecogeographical gradient in Israel and the West Bank *Mol. Ecol.* **11**(1), 39-53.

Atlan, A., *Barat*, M., and A. S. *Legionnet* (2010). Genetic variation in flowering phenology and avoidance of seed predation in native populations of Ulex europaeus. *J. Evol. Biol.* **23**, 362-371.

Avramov, *S.* (1995). Genetička analiza ranih stupnjeva razvića *Iris pumila* L. (Iridaceae). Univerzitet u Beogradu MSci thesis (in Serbian).

Avramov, *S.* (2008). Ontogenetska fenotipska plastičnost i materinski efekti tokom ranih stunjeva razvića *Iris pumila* L. (Iridaceae). Univerzitet u Beogradu Ph.D thesis (in Serbian).

Avramov, *S.,* and *B. Tucić* (2006). Plasticity to an irradiance gradient in foliage attributes of a perennial monocot *Iris pumila* (L.): Comparison of populations from habitats of contrasting light conditions *Pol. J. Ecol*. **54**, 403-416.

Avramov, *S., Pemac*, *D.*, and *B. Tucić* (2007). Phenotypic plasticity in response to an irradiance gradient in Iris pumila: adaptive value and evolutionary constraints *Plant Ecol.* **190**, 275-290.

Back, *A. J., Kron*, *P.* and *S.C. Stewart* (1996). Phenological Regulation of Opportunities for within-Inflorescence Geitonogamy in the Clonal Species, *Iris versicolor* (Iridaceae). *Am. J. Bot*. **83**, 1033-1040.

Badyaev, A. V., and *K. R. Foresman* (2000). Extreme environmental change and evolution: stress-induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. *Proc. Biol. Sci.* **267**, 371-7.

Barišić Klisarić, *N., Avramov*, *S., Miljković, D*., *Živković*, *U.,* and *A. Tarasjev* (2011). Morphometry in flower bud stage on naturally growing *Iris pumila* clones: Implications for population differentiation and phenotypic plasticity studies XIX DFBS Symposium.

Baythavong, *B.S.,* and *M.L. Stanton* (2010). Characterizing selection on phenotypic plasticity in response to natural environmental heterogeneity. *Evolution* **64**(10), 2904-2920.

Bell, *D. L.,* and *L. F. Galloway* (2008). Population differentiation for plasticity to light in an annual herb: adaptation and cost. *Am. J. Bot* **95**, 59-65.

Buide, M.L., Diaz-Peromingo J.A., and *J.Guitian* (2002). Flowering phenology and female reproductive success in *Silene acutifolia* Link ex Rohrb. *Plant.Ecol.* **163**(1), 93-103.

ColladoVides, *L.* (1997). Consecuencias ecológicas y evolutivas de la arquitectura modular en plantas: Perspectivas en algas marinas. *Rev. Chil.Hist.Nat* **70**, 23-29.

Cottrell, *J.E., Vaughan*, *S.P.,* and *T. Connolly* (2009). Contemporary pollen flow and mating patterns in wild cherry (*Prunus avium* L.). *Heredity* **103** (2), 118-128.

Cuervo, J. J., and *A. P. Moller* (1999). Phenotypic variation and fluctuating asymmetry in sexually dimorphic feather ornaments in relation to sex and mating system. *Biol. J. Linn. Soc*. **68**, 505-529.

Donovan, *L. A., Maherali*, *H.*, *Caruso*, *C. M., Huber*, *H.*, and *H.* de *Kroon*. (2011). The evolution of the worldwide leaf economics spectrum. *Trends in Ecology & Evolution* **26(**2), 88-95.

Dutilleul, *P.*, and *C. Potvin* (1995). Among-environment heteroscedasticity and genetic autocorrelation: Implications for the study of phenotypic plasticity. *Genetics* **139**, 1815-1829.

Ernande, *B.*, and *U. Dieckmann* (2004). The evolution of phenotypic plasticity in spatially structured environments: Implications of intraspecific competition, plasticity costs and environmental characteristics. *J. Evol. Biol*. **17**, 613-628.

Esser, *K.*, *Luttge*, *U.*, *Beyschlag*, *W.*, and *F. Hellwig* (2002). *Progress in Botany*. Springer.

Franiel, *I.*, and *K. Więski*, (2005). Leaf features of silver birch (*Betula pendula* Roth). Variability within and between two populations (uncontaminated vs Pb-contaminated and Zn-contaminated site). *Trees - Structure and Function* **19**(1), 81-88.

Forde, *G. B.* (2009). Is it good noise? The role of developmental instability in the shaping of a root system. *J. Exp. Bot.* **60**, 3989-4002.

Fu, *W.*, *Yao*, *J.*, *Wang*, *X.*, *Liu*, *F.*, *Fu*, *G.*, and *D. Duan* (2009). Molecular cloning and expression analysis of a cytosolic Hsp70 gene from *Laminaria japonica* (Laminariaceae, Phaeophyta). *Mar. Biotech.* **11**(6), 738-747.

Futyuma, *D. J.* (1998). Evolutionary Biology, 3rd Ed. Sinauer Sunderland, Massachusetts.

Henkel, *S.K.* and *G.E. Hofmann* (2008). Differing patterns of *hsp70* gene expression in invasive and native kelp species: Evidence for acclimation-induced variation. *J App Phyc* **20**, 915–924.

Henkel, *S.K.*, *Kawai*, *H.* and *G.E. Hofmann* (2009). Interspecific and interhabitat variation in *hsp70* gene expression in native and invasive kelp populations. *MEPS* **386**, 1–13.

Imbert, *E.*, *Escarré*, *J.* and *J. Lepart* (1999). Local adaptation and non-genetic maternal effects among three populations of *Crepis sancta* (Asteraceae). *Ecoscience* **6**, 223-229.

Kellog, E.A., and *H.B.* Shaffer (1993). Model organisms in evolutionary studies. *Syst Biol* **42**(4), 409–414.

Kostrakiewicz, *K.* (2007). The effect of dominant species on numbers and age structure of *Iris sibirica* L. population on blue moorgrass meadow in southern Poland. *Acta. Soc. Bot. Pol.* **76**, 165–173.

Kristensen, *T.N., Pertoldi*, *C.*, *Andersen, D.H.*, and V. *Loeschcke* (2003). The use of fluctuating asymmetry and phenotypic variability as indicators of developmental instability: a test new method employing clonal organisms. *Evol. Ecol. Res.* **5**, 53-68.

Laugen, *A. T.*, *Laurila*, *A.*, and *J. Merilä* (2002). Maternal and genetic contributions to geographic variation in *Rana temporaria* life histories. *Biol. J. Linn. Soc.* **76**, 61-70.

Lepik, *M.*, *Liira*, *J.,* and *K. Zobel* (2004). The space-use strategy of plants with different growth forms, in a field experiment with manipulated nutrients and light. *Fol. Geobot.* **39**, 113-127.

Ley, A. C., and *O. J. Hardy* (2010). Species delimitation in the Central African herbs *Haumania* (Marantaceae) using georeferenced nuclear and chloroplastic DNA sequences. *Mol. Phylogen. Evol.* **57** (2), 859- 867.

Liao, *W. J.*, *Hu*, *Y.*, and *B. R. Zhu* (2009). High variation in clonal vs. sexual reproduction in populations of the wild strawberry, Fragaria virginiana (Rosaceae). *Ann. Bot.* **104(**7), 1405-1412.

Lind, *M. I.,* and *F. Johansson* (2009). Costs and limits of phenotypic plasticity in Island populations of the common frog *Rana temporaria* under divergent selection pressures. *Evolution.* **63** (6), 1508-1518.

Loranger, *J.*, and *B. Shipley* (2010). Interspecific covariation between stomatal density and other functional leaf traits in a local flora. *Botany* **88** , 30-38.

Mandoli, *D.F.,* and *R.G. Olmstead* (2001). The importance of emerging model systems in plant biology. *J. Pl. Growth Regulators* **19**, 249-252.

Manitašević, S. (2010). Ekološko-evolucioni aspekti ekspresije protena toplotnog stresa HSP70 i HSP90 u prirodnim populacijama *Iris pumila* L Univerzitet u Beogradu Ph.D thesis (in Serbian).

Manitašević, S., *Dunđerski*, *J.*, *Matić*, *G.,* and *B. Tucić* (2007). Seasonal variation in heat shock proteins Hsp70 and Hsp90 expression in an exposed and a shaded habitat of Iris pumila. *Plant Cell Environ.* **30**, 1-11.

Manitašević-Jovanović, *S.*, *Dunđerski*, *J., Matić, G.,* and *B. Tucić* (2011). Differential expression of heat-shock proteins Hsp70 and Hsp90 in vegetative and reproductive tissues of *Iris pumila. Acta Physiol. Plant.* **33** (1), 233-240.

Martinkova, *J.*, *Smilauer*, *P.*, and *S. Mihulka* (2002). Phenological pattern of grassland species: relation to the ecological and morphological traits. *Flora* **197** (4), 290-302.

Mathew, *B.* (1981). The Iris. Published by Timber Press, Portland, Oregon.

Miljković. D. (2009). Uticaj abiotičkih stresora na stabilnost razvića i morfološku varijabilnost *Iris pumila* u prirodnim i eksperimentalnim populacijama.Univerzitet u Beogradu Ph.D thesis (in Serbian).

Miljković, *D.*, *Avramov*, *S.*, *Živković, U.*, *Barišić Klisarić*, *N.*, and *A. Tarasjev* (2011). Physiological traits in early and late stages of *Iris pumila* development: genetic analysis and analysis of correlations. XIX DFBS Symposium.

Miljković. D. (2011). Developmental instability of *Iris pumila* floral traits. XIX DFBS Symosium.

Møller, A. P., and *J. P*, *Swaddle* (1997). *Asymmetry, Developmental Stability and Evolution,* Oxford Series in Ecology and Evolution.

Monty, A., *Saad, L.*, and *G. Mahy* (2006). Bimodal pollination system in rare endemic *Oncocyclus irises* (Iridaceae) of Lebanon. *Can. J. Bot*. **84**, 1327-1338.

Navas, *M. L.*, and *E. Garnier* (2002). Plasticity of whole plant and leaf traits in *Rubia peregrina* in response to light, nutrient and water availability. *Acta Oecol.* **23**, 375-383.

Obeso, J.R., and *C.M. Herrera* (1994). Inter- and intraspecific variation in fruit traits in co-occurring vertebrate-dispersed plants. *Int. J. Plant. Sci*. **155**, 382-387.

Obrdlik, *P.*, and *L. C. Garcialozano* (1992). Spatio-temporal distribution of macrozoobenthos abundance in the Upper Rhine alluvial floodplain. Archiv für Hydrobiologie. *Arch. Hydrobiol.* **124,** 205-224.

Palmer, *R.*, and *C. Strobeck*, (2003). Fluctuating asymmetry analyses revisited, in *Developmental Instability (DI): Causes and Consequences*, M. Polak, ed. Oxford University Press, Oxford.

Pelabon, C., Armbruster, *W. S.*, and *T. F. Hansen* (2011). Experimental evidence for the Berg hypothesis: vegetative traits are more sensitive than pollination traits to environmental variation. *Func. Ecol.* ***25***(1), 247- 257.

Pemac, D. (1997) Norme reakcije juvenilnih osobina *Iris pumila* u odnosu na intenzitet svetlosti PMF. Univerzitet u Beogradu M.Sci thesis (in Serbian).

Pemac, D., and *B. Tucić* (1998). Reaction norms of juvenile traits to light intensity in *Iris pumila* (Iridaceae): a comparison of populations from exposed and shaded habitats. *Pl. Sys. Evol*. **206**, 133-159.

Pico, F. X., and *J. Retana* (2001). The flowering pattern of the perennial herb Lobularia maritima: an unusual case in the Mediterranean basin. *Acta Oecol.* **22**(4), 209-217.

Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture*. John Hopkins University Press.

Price, *M. W.*, and *N. M. Wasser* (1998). Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology* **79**(4), 1261-1271.

Ramírez-Valiente, *H. A., D. Sánchez-Gómez*, *I. Aranda*, and *F. Valladares* (2010). Phenotypic plasticity and local adaptation in leaf ecophysiological traits of 13 contrasting cork oak populations under different water availabilities. *Tree Physiol.* **30**(5), 618-627.

Reusch, *T.*, and *W. U. Blanckenhorn* (1998). Quantitative genetics of the dung fly Sepsis cynipsea: Cheverud's conjecture revisited. *Heredity* **81**, 111-119.

Roff, *D. A.*, and *D. J. Fairbairn* (2007). The evolution of trade-offs: where are we? J. Evol. Biol. **20**, 433-447.

Roncal, *J.*, *Francisco-Ortega*, *J. A.*, and *C. E. Lewis* (2007).An evaluation of the taxonomic distinctness of two *Geonoma macrostachys* (*Arecaceae*) varieties based on inter simple sequence repeat (ISSR) variation. *Bot. J. Linn. Soc*. **153**, 381-392.

Roy, B. A., and *M. L. Stanton* (1999). Asymmetry of wild mustard, *Sinapsis arvensis* (*Brassicaceae*), in response to severe physiological stresses. *J. Evol. Biol*. **12**, 440-449.

Sapir, *Y.*, *Shmida*, *A.*, *Fragman*, *O.,* and *H. P. Comes* (2002).Morphological variation of the *Oncocyclus* irises (Iris:Iridaceae) in the southern Levant. *Bot. J. Linn. Soc.* **139**, 369-382.

Schlichting, *C. D.* (2008). Hidden reaction norms, cryptic genetic variation, and evolvability. *Annals of the New York Academy of Sciences* **1133**, 187–203.

Schlichting, *C. D.,* and *M. Pigliucci* (1998). *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer Associates Inc.

Schlichting, *C. D.,* and *H. Smith* (2002). Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes. *Evol. Ecol.* **16**, 189-211.

Steinger, *T.*, *Roy*, *B. A.*, and *M.L. Stanton* (2003). Evolution in stressful environments II: adaptive value and costs of plasticity in response to low light in *Sinapis arvensis*. *J. Evol. Biol.* **16**, 313-323.

Stjepanović-Veseličić, *L.* (1953): Vegetacija Deliblatske peščare. SAN. Posebno izdanje knjiga CCXVI. Institut za ekologiju I biogeografiju, knjiga **4**, str 1-113. Beograd.

Sultan, *S. E.* (2001). Phenotypic plasticity for fitness components in polygonium species of contrasting breadth. *Ecol.* **82** (2), 328-343.

Tagane, *S*., *Hiramatsu M*., and *H. Okubo* (2007). Lack of prezygotic reproductive isolation between *Rhododendron eriocarpum* and *R. indicum*; Overlapping flowering phenology and cross compatibility. *J. Fac. Agr. Kyushu Univ.* **52**, 345-348.

Tamás, J. and *L. Hably* (2005). The morphometrical analysis of *Sloanea elliptica* (Andreanszky) Z. Kvacek & Hably from the Oligocene of the Buda and Bukk Mountains and its palaeogeographic implications *Rev. Palaeobot. Palynol*. **134**, 257-268.

Tamás, J. and *L. Hably* (2009). Unidirectional shift in leaf morphology of coexisting species - a possible indicator of palaeoclimatic differences. *Palaeogeog., Palaeoclim, Palaeoecol.* Elsevier, **271**(3–4), 185–195.

Tarasjev, *A*. (1989). Genetička osnova fenotipske plastičnosti i morfološka diferencijacija populacija *Iris pumila* L (Iridaceae) PMF. Univerzitet u Beogradu M.Sci thesis (in Serbian).

Tarasjev, *A*. (1994). Plastic response to between-year environmental variability in populations and subpopulations of *Iris pumila.* L. *Arch. Biol. Sci.* **3-4**, 105-114.

Tarasjev, *A*. (1995a). Fenotipska plastičnost u prirodnim populacijama *Iris pumila* L Biološki fakultet. Univerzitet u Beogradu Ph. D thesis (in Serbian)

Tarasjev, A. (1995b). Relationship between phenotypic plasticity and developmental instability in *Iris pumila.* L. *Russ. J. Genet.* **31**(12), 1655-1663.

Tarasjev, *A*. (1997). Flowering phenology in natural populations of *Iris pumila*. *Ecography* **20**: 48-54.

Tarasjev, *A*. (2002). Variability of life history traits in *Iris pumila* juvenile plants: ramet dynamics. *Russ. J. Genet* **38**(11), 1492-1497.

Tarasjev, *A*. (2003). Relationship between estimates of spatial and temporal plasticity, genetic variability and between-population differentiation in *Iris pumila*. *Arch. Biol. Sci*. **55**, 5P-6P.

Tarasjev, A. (2005a). Impact of genet size and flowering stage on fruit set in *Iris pumila* L. clones in wild. *Acta Oecol* 27(2): 93-98

Tarasjev, *A*. (2005b). Variation in survival of *Iris pumila* L. juvenile plants grown in two nutrient regimes. *Russ. J. Genet* **41**(2), 211-213.

Tarasjev, *A*. (2005c). Evolutionary ecology of dwarf bearded iris (*Iris pumila*) in Deliblato Sand –Annotated bibliography. *Deliblatska Peščara VII* , Vojvodinašume str 321-29.

Tarasjev, *A. Barišić- Klisarić, N*., and *B. Stojković* (2006). Spatial autocorrelation in two *Iris pumila* populations estimated on morphological data from natural clones and their samples grown in two different habitats. *Russ. J. Genet*. **42**(2), 215–218.

Tarasjev, *A*., *Barišić- Klisarić, N*., and *Stojković, B*., and *S. Avramov* (2009). Phenotypic plasticity and between population differentiation *Iris pumila* transplants between native open and anthropogenic shade habitats. *Russ. J. Genet*. **45**, 944-952.

Tonsor, S. J., *Scott, C. M*., *Boumaza, I*., *Liss, T. R*., *Brodsky, J. L*., and *E. Vierling* (2008). Heat shock protein 101 effects in *A. thaliana*: genetic variation, fitness and pleiotropy in controlled temperature conditions. *Mol. Ecol.* **17**, 1614-26.

Torres, E., *Iriondo, J. M.,* and *C. Perez* (2002). Vulnerability and determinants of reproductive success in the narrow endemic *Antirrhinum microphyllum* (Scrophulariaceae). *Am. J. Bot*. **89** (7), 1171-1179.

Tucić, *B*., *Rak-Šoltes*, *E*., and *V*., *Stevanović* (1984). Genetic distance and organization of isoenzyme variation in several Iris species. *Biosistematika* **10**, 13–23.

Tucić, *B*., *Rak-Šoltes*, *E*., and *N. Tucić* (1984). Isozyme variability in the *Iris* species. *Arch. Biol Sci.* **36**, 1–12.

Tucić, *B*., *Milojković, S*., *Vujčić*, *S*., and *A. Tarasjev* (1988). Clonal diversity and dispersion in *Iris pumila*. *Acta Oecol/Oecol Plant.* **8**, 211-219.

Tucić, *B*., *Milojković*, *S*., *Tarasjev*, *A*., and *S.Vujčić* (1989). The influence of climatic factors on clonal diversity in a population of *Iris pumila*. *Oikos* **56**, 115-120.

Tucić, *B*., *Vujčić*, *S*., *Tarasjev*, *A*., and *S. Milojković* (1989). Population growth of *Iris pumila* L . - a rhizomatous clonal herb. *Arch. Biol. Sci.* **41**, 159-165.

Tucić, *B*., *Tarasjev*, *A*., *Vujčić*, *S*., *Milojković*, *S*., and *N. Tucić* (1990). Phenotypic plasticity and character differentiation in a subdivided population of *Iris pumila* (Iridaceae). *Plant. Sys. Evol*. **170**, 1-9.

Tucić, B., and *S. Avramov* (1996a). Maternal effects on early juvenile traits in *Iris pumila* (Iridaceae). *Pl. Sys. Evol.* **201**, 179-197.

Tucić, B., and S. *Avramov* (1996b). Morphological integration in the seedlings of Iris *pumila* L. *Russ. J. Gen*. **32**, 1235-1242.

Tucić, B, *Tomić*, *V*., *Avramov*, *S*. and *D. Pemac* (1998). Testing the adaptive plasticity of *Iris pumila* leaf traits to natural light conditions using phenotypic seleciton analysis. *Acta Oecol.* **19**(6), 473-481.

Tucić, *B*., *Pemac*, *D*., *Stojković*, *B*., and *S. Avramov* (1999). Coping with environmental changes in *Iris pumila*: a pilot experiment. *Arch. Biol. Sci.* **51**, 137–148.

Tucić, *B*., *Pemac*, *D*., and S. *Avramov* (2003). Plasticity to daylength of *Iris pumila* leaf phenological traits. *Popul. Ecol.* **45**(1), 31-39.

Tucić, *B*., *Pemac, D*., and *S. Avramov* (2005). Testing the predictions of an evolutionary trade-off model using *Iris pumila* plants from an open and a shaded habitat. *Plant Spec. Biol.* **20**(1), 17-22.

Tucić, *B*., *Manitašević, S*., and *A. Vuleta* (2008). Linking Hsp90 function to micro-environmental and stochastic variation in floral organs of *Iris pumila*. *Arch. Biol. Sci.* **60**, 411-419.

Tucić, *B*., *Vuleta*, *A*., and *S. Manitašević-Jovanović* (2009). Protective function of foliar anthocyanins: in situ experiments on a sun-exposed population of *Iris pumila* L. (Iridaceae). *Pol. J. Ecol.* **57**, 779-783.

Tucić, *B*., and *D. Miljković* (2010). Fluctuating asymmetry of floral organ traits in natural populations of *Iris pumila* from contrasting light habitats. *Plant. Spec. Biol.* **25**, 173-184.

Valladares, F., *Sánchez, D*., and *M. A. Zavala* (2006). Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *J. of Ecol*. **94**(6), 1103–1116.

Vallejo-Mar, *M*., *Dorken*, *M. E*., and *S. C. H. Barrett* (2010). The ecological and evolutionary consequences of clonality for plant mating. *Ann. Rev. Ecol. Evol. Syst*. **41**, 193-213.

Van Buskirk, J., and *U. K. Steiner* (2009). The fitness costs of developmental canalization and plasticity. J. Evol. Biol. **22**(4), 852-860.

Van Kleunen, M., and *M. Fisher* (2005). Constraints on the evolution of phenotypic plasticity in plants. *New Phytol*. **166**(1), :49-60.

Vasseur, L., *Aarssen, L.W*. and *D. D. Lefebvre* (1991). Allozymic and morphometric variation in *Lemna minor* (Lemnaceae). *Pl. Sys. Evol*. **177**, 139-148.

Vuleta, *A*., and *B. Tucić* (2009). Thermal dependence of the antioxidant enzymes superoxide dismutase, catalase, and peroxidase in foliage of *Iris pumila* L. *Arch. Biol. Sci.* **61**(3), 441-446.

Vuleta, *A*., *Jovanović*, *S. M*., *Šešlija*, *D*., and *B. Tucić* (2010). Seasonal dynamics of foliar antioxidative enzymes and total anthocyanins in natural populations of *Iris pumila* L. *J. Plant. Ecol.* **3** SI, 59-69.

Wolfe, L. M. and *S. J. Mazer* (2005). Patterns of phenotypic plasticity and their fitness consequences in wild radish (*Raphanus sativus*: Brassicaceae). *Int. J. Plant Sci.* **166**, 631 – 640.

Živković, *U*., *Barišić Klisarić*, *N*., *Avramov*, *S*., *Miljković*, *D*., *Kostić M., Kecojević I.,* and *A. Tarasjev* (2011). Analyses of *Iris sp.* from localities and populations in polluted and unpolluted environments. XIX DFBS Symposium.

Zhao, Y., *Qing*, *J. H., Zhao*, *C. J*., *Zhou*, *C. F*., *Zhang W.G.,* and *Y. Xiao* (2010). Phenotypic plasticity of Spartina alterniflora and Phragmites australis in response to nitrogen addition and intraspecific competition. *Hydrobiologia* **637**, 143–155.